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Detection of Mitotic and Meiotic Aneuploidy in the Yeast *Saccharomyces cerevisiae*

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A number of genetic systems are described which involve the use of the yeast *Saccharomyces cerevisiae*. The systems may be used to detect the production of aneuploid cells produced during both mitotic and meiotic cell division in the presence of genetically active chemicals.

During mitotic cell division, monosomic colonies ($2n - 1$) may be detected by plating upon selective medium. Increases in such monosomic colonies are produced by exposure of cells to a number of chemical mutagens such as ethyl methane-sulfonate and mitomycin C. More importantly, monosomic colonies are also induced by nonmutagens such as sulfacetamide and saccharin, which suggests that such chemicals are capable of inducing aneuploidy (aneugenic) in the absence of mutagenic activity. Genetic analysis of aneuploid colonies produced on nonselective medium indicate that at least a proportion of the monosomic colonies were the result of mitotic nondisjunction.

During meiotic cell division, disomic cells ($n + 1$) produced by chromosome nondisjunction may be detected by plating on selective media. The frequency of disomic cells has been shown to increase after exposure to *p*-fluorophenylalanine.

Introduction

The processes of cell division meiosis and mitosis must give exact distributions of chromosomes or chromatids to daughter cells if the chromosome number and genetic integrity of a species is to be maintained as a constant. However, these processes may be disturbed and the resulting cells may be euploid or aneuploid. Euploidy describes chromosome numbers which are multiples of the haploid number, whereas aneuploidy describes chromosome numbers which are not multiples of the haploid number.

Aneuploid cells can result from nondisjunction, which is the failure of paired chromosomes or sister chromatids to pass to opposite poles of the spindle at the anaphase of meiosis or mitosis. In mitosis such nondisjunction will result in one trisomic daughter cell and one monosomic daughter cell. Aneuploidy may also result from anaphase lagging, in which chromosomes or chromotids separate normally in the early stages of cell division but one chromosome or chromatid moves slowly to the spindle pole and tends to be included in the nucleus of neither daughter cell. In mitosis this would result in one daughter cell being a normal diploid and the other a monosomic. In multicellular organisms mitotic nondisjunction may give rise to mosaicism: it may occur during embryonic development or at later stages; the latter may be associated with increasing age (*1, 2*) or neoplastic change (*3*).

Aneuploidy resulting from meiotic nondisjunction usually gives such a gross imbalance of chromosomes as to be fatal. In man, many types of autosomal aneuploidy have been found in spontaneously aborted embryos, but only trisomic conditions of some of the smaller autosomes or the sex chromosomes are compatible with postnatal life unless in a mosaic form (*4*). Such surviving aneuploids represent a considerable individual and social burden. Three autosomal trisomies are well established in man: trisomy 21, 18, and 13; all three show an increased mean maternal age at birth (*4*). It is possible that differences in the meiotic pattern in the two sexes may account for this. Paired meiotic chromosomes are held together by chiasmata, structures

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which are essential for the co-orientation of bivalents at first meiotic metaphase. The longer this period (in females from the fetal ovary until ovulation), the greater the risk of disrupting their integrity, either as a result of an ageing process or from environmental influences.

The yeast *Saccharomyces cerevisiae* is stable in either a haploid phase or a diploid phase, although in nature the diploid phase may predominate. Aneuploid states also appear to be quite stable; both diploids monosomic ($2n - 1$) for one chromosome and haploids disomic ($n + 1$) have been found to be stable enough for genetic studies and repeated culturing (*5-16*). Many fungi have a parasexual cycle, the main feature of which is the progressive haploidisation of diploid nuclei which is believed to occur through successive nondisjunctional events (*17*). In such fungi the haploid stage is the stable form in nature. In contrast, in yeast Strömnaes (*18*) has shown that DL-*p*-fluorophenylalanine treatment of a diploid cell induces the formation of monosomics, but that haploidisation does not occur. He also found that many of his observations could best be explained by repeated nondisjunctional events restoring diploidy. Since the vegetative cells of *Saccharomyces cerevisiae* are normally diploid, we would expect there to be stronger selection in a monosomic ($2n - 1$) cell for nondisjunctional events leading back to diploidy than towards haploidy because of the relative inviability of aneuploids. In mycelial fungi, unstable nuclei or nuclei with poor viability may survive in multinucleate hyphae until a stable form is achieved. Successive nondisjunctional events may lead from a diploid nucleus to aneuploids and to a stable haploid form, but in a single-cell organism like yeast it is not expected that successive nondisjunction will occur to haploidy. In this respect (preference for the diploid state), yeast studies may have more relevance to our understanding of nondisjunction in higher organisms.

Meiosis and mitosis are themselves under genetic control, and some of the genes involved may have an effect upon the production of aneuploids. In yeast, Haber (*15*, *19*) has demonstrated a gene controlling chromosome loss of chromosome III and possibly of others. Culbertson and Henry (*11*) have described a fatty-acid requiring mutant in *Saccharomyces cerevisiae* which exhibits a high frequency of spontaneous aneuploidy: the effect appears to be associated with the chromosome on which the gene is mapped. In *Drosophila melanogaster* mitotic instability has also been reported; examples are claret nondisjunction (*20*, *21*), paternal loss (*22*), and mitotic loss inducer (*23*). Mutants having an effect on meiotic aneuploidy in Drosophila have also been reported (*24-26*). Davis (*24*) described one which causes a high frequency of nondisjunction of all chromosomes at the second division of meiosis in males and females apparently by the precocious separation of sister centromeres so that sister chromatids are not directed to opposite poles at metaphase.

Campbell, Fogel, and Lusnak (*16*) have studied mitotic chromosome loss of chromosome III in yeast and claim a correlation between chromosome loss and genetic exchange. They propose that such exchange may potentiate sister centromeres to separate prematurely.

Thus it is well established that aneuploidy occurs spontaneously in yeast; Campbell, Fogel, and Lusnak (*16*) give a value in the order of 10^{-4}/cell for the loss of chromosome III during mitosis. Parry and Zimmermann (*27*) have studied the effects of physical agents upon the rate of aneuploidy of chromosome VII. The chromosome was heavily marked along its length and on both sides of the centromere so that, should nondisjunction occur, all genetic markers on this chromosome segregate *en bloc*. They found that ultraviolet, x-rays, and heat treatment were all effective inducers of aneuploidy during mitotic cell division.

Of necessity, aneuploidy of one chromosome is usually monitored at a given time; but Strömnaes (*18*) and Parry and Cox (*28*) have reported that it is common for aneuploids to be monosomic for more than one chromosome. Similarly, Parry and Zimmermann (*27*) reported that after treatment with ultraviolet light at least a proportion of the colonies detected which were monosomic for chromosome VII appeared to be aneuploid for other chromosomes. Moreover, Parry and Cox (*28*) and Bruenn and Mortimer (*5*) suggest a differential survival of aneuploids depending upon which specific chromosome is monosomic.

In this paper we describe a number of genetic systems which have been developed in yeast which are capable of detecting the induction of aneuploid cells produced during both mitosis and meiosis. These strains have been used to screen a wide range of environmental chemicals for possible activity in inducing aneuploidy.

Materials and Methods

Detection of Aneuploid Cells Produced during Mitotic Cell Division.

The diploid strain of *Saccharomyces cerevisiae* used for the detection of monosomic colonies ($2n - 1$) produced by chromosome loss during mitosis was *D6*, the genetic details of which have been fully described elsewhere (*27*). This yeast strain carries a series of recessive and coupled markers on chromo-

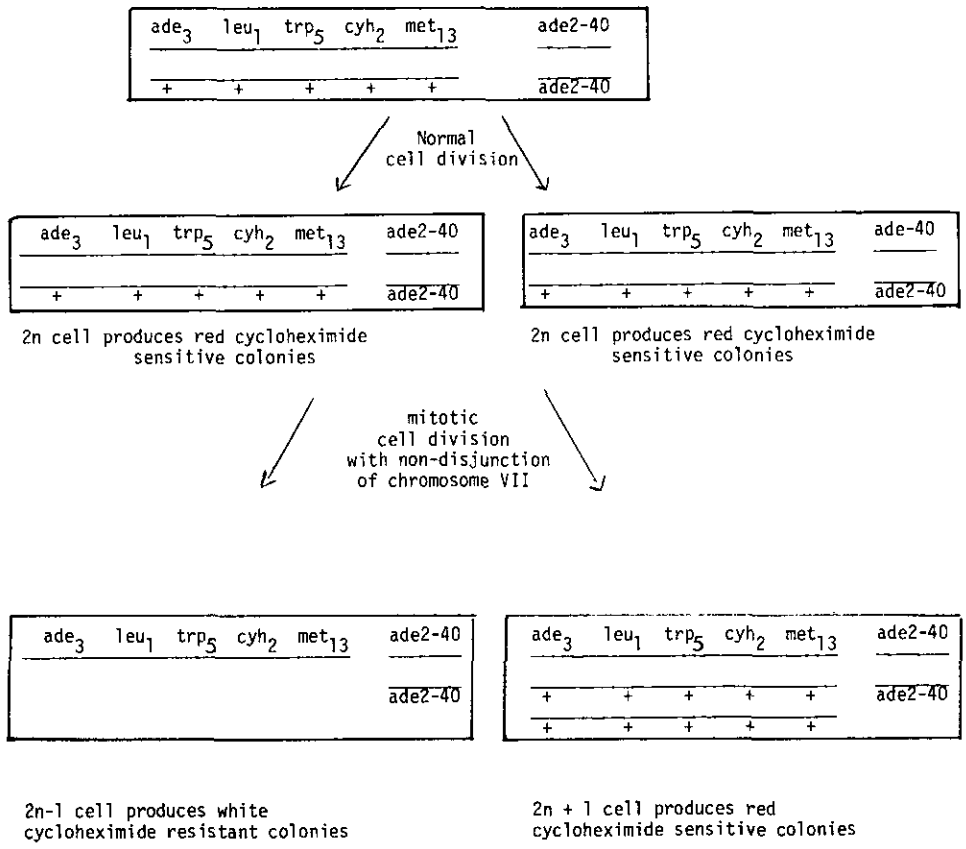

FIGURE 1. Outline of the genetic principles involved in the detection of aneuploid ($2n - 1$) cells by using the yeast strain *D6*.

some VII. The strain produces red colonie due to the presence of the defective alleles *ade2-40* of the gene *adenine-2* in a homozygous condition on chromosome XV, which require adenine for growth and are sensitive to the presence of 2 mg/l. of cycloheximide in the growth medium. The loss of the copy of chromosome VII carrying the wild type markers results in the production of cells carrying only the recessive and mutant alleles of the five genes shown in Figure 1. The resulting monosomic cells ($2n - 1$) are white in color due to the presence of the defective allele of the gene *adenine-3* which results in a mutant block in the adenine synthetic pathway prior to the *adenine-2* gene, thus preventing formation of the red pigment characteristic of cells carrying defective alleles of this latter gene. Monosomic colonies were also characterized by their resistance to cycloheximide.

The genotype of *D6* is:

$$\text{Chromosome III } \frac{\text{his 4}}{+} \text{ centromere } \frac{a}{\alpha} \text{ chromosome XV } \frac{\text{ade 2-40}}{\text{ade 2-40}}$$

$$\text{Chromosome VII } \frac{\text{ade}_3 +}{+ \text{ ade}_6} \text{ centromere } \frac{\text{leu}_1\ \text{trp}_5\ \text{cyh}_2\ \text{met}_{13}}{+\ +\ +\ +}$$

The principles involved in the detection of monosomic ($2n - 1$) colonies in *D6* are outlined in Figure 1.

In order to detect the production of monosomic colonies in *D6*, two different procedures have been used. In both procedures, cells treated with test chemicals are allowed to undergo a number of cell divisions to allow the events leading to aneuploidy to take place in nonselective medium. The requirement for this period of nonselective growth has been demonstrated previously after ultraviolet and x-ray treatment (*27*). In some experiments, cells were treated with test chemical before innoculation into growth media whereas in other experiments the test chemical was present during the whole of the growth period in nonselective medium.

Test Procedure 1. Samples of 10 ml of liquid yeast complete medium were innoculated with 10^5 yeast cells harvested from a stationary phase culture. The

samples were grown for 3 to 5 days in conical flasks with aeration on an orbital shaker at 28°C, during which time cell numbers reach approximately 2×10^7 cells/ml. Cells were harvested by centrifugation and washed 3x in sterile saline. After appropriate dilution, samples were plated on solid yeast complete medium to score viability and upon solid yeast complete medium plus 2 mg/l. of cycloheximide (Koch-Light) to score the frequency of white, cycloheximide-resistant, monosomic colonies.

Test Procedure 2. This procedure is illustrated in Figure 2 and has been developed by us specifically for the routine testing of chemicals. In this technique, cells were grown in 2 ml of 1/5 normal strength liquid yeast complete medium supplemented with 2 mg/l. adenine and histidine (to prevent selective effects during growth) in sealed disposable bottles. The bottles contain an appropriate dose range of the test chemical with or without an S9 extract plus the appropriate cofactors and were incubated with shaking for 18 hr at 30°C, during which time the cells undergo approximately four cell divisions. Cells from the treated bottles were plated (without washing) after appropriate dilution on solid complete medium to score viability and upon solid complete medium plus 2 mg/l. of cycloheximide to score the frequency of white, cycloheximide-resistant colonies.

In both procedures, control cultures which failed to produce white cycloheximide-resistant colonies were rejected because of the occasional production by mitotic crossing-over of homozygous ade-*3* cultures which fail to produce white colonies by chromosome loss.

Detection of Disomic spores ($n + 1$) Produced by Nondisjunction during Meiotic Cell Division in *Saccharomyces cerevisiae*

The strain used for the detection of disomic spores ($n + 1$) produced by nondisjunction during meiosis was D_9J_2. This strain is a derivative of D_9 originally

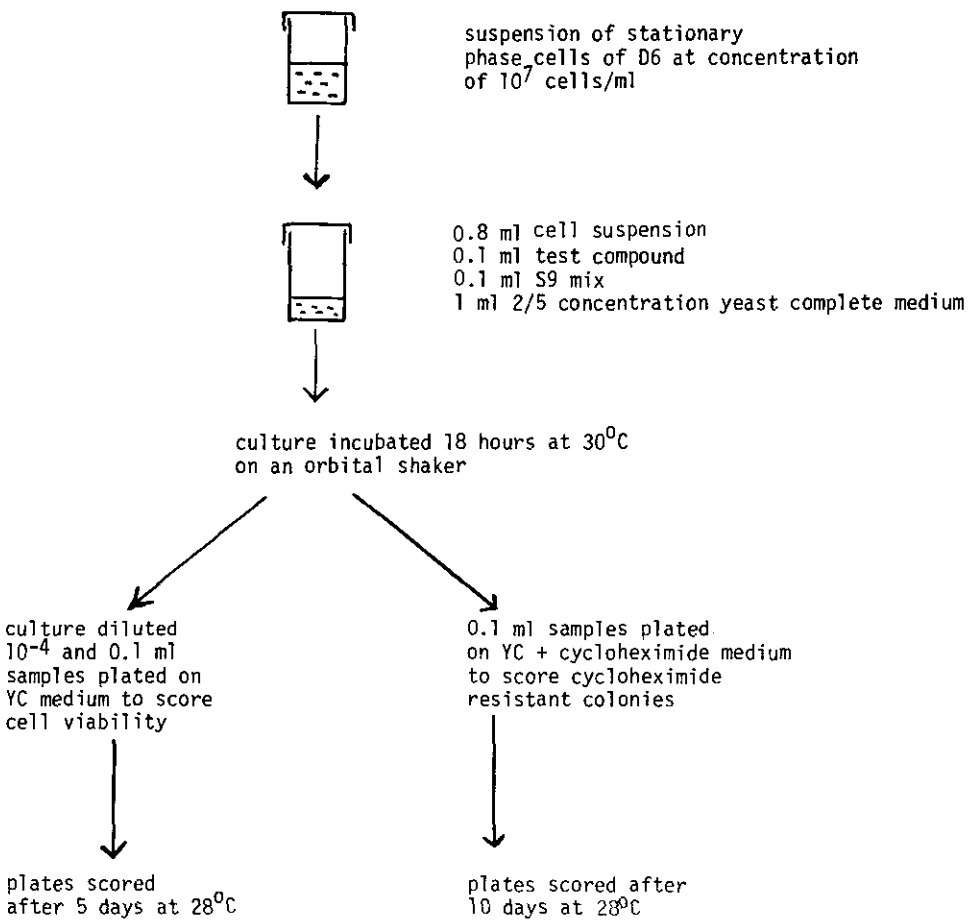

FIGURE 2. Flow sheet to illustrate the technical procedure 1 used to detect aneuploid ($2n - 1$) cells in strain *D6*.

constructed by f. Zimmermann. The strain carries a series of recessive markers in repulsion on both arms of chromosome VII. The genotype of D_9J_2 is:

division of meiosis. The system used in our experiments is not capable of detecting disomic spores produced during the second division of meiosis by

$$\begin{array}{lll} \text{Chromosome V} & \underline{\text{can}_1} & \text{Chromosome XI} \quad \underline{\text{ura}_1} \\ & + & \quad + \\ \text{Chromosome VII} & \underline{\text{ade}_3 +} \quad \text{centromere} & \underline{\text{leu}_1 + \text{cyh}_2 + \text{aro}_2 + \text{ade}_5} \\ & + \text{ade}_6 & + \text{trp}_5 + \text{met}_{13} + \text{lys}_5 + \end{array}$$

Diploid cultures of D_9J_2 grow on solid yeast minimal medium but are sensitive to the presence of 30 mg/l. of canavanine (Sigma) in the medium.

Haploid spores of D_9J_2 produced by meiotic cell division in acetate sporulation medium have multiple growth requirements (each copy of chromosome VII confers a requirement for at least four nutrients) and will not grow on solid yeast minimal medium. Disomic spores (n + 1) carrying multiple copies of chromosome VII are capable of growth on minimal medium containing 10 mg/l. uracil, if it is assumed the nondisjunctional event occurred during the first plating on selective medium. Of the disomic spores produced, 50% are capable of growth on minimal medium containing uracil and canavanine which selectively kills residual diploid cells which have not undergone meiosis. The principles of the genetic system used to detect spores are outlined in Figure 3.

In the treatment procedure used with strain D_9J_2 for the measurement of disomic spores, cells were grown stationary phase, washed 3 × in saline and resuspended in acetate sporulation medium at a concentration of 10^7 cells/ml. The cells were either treated with test agent at this stage and the cells

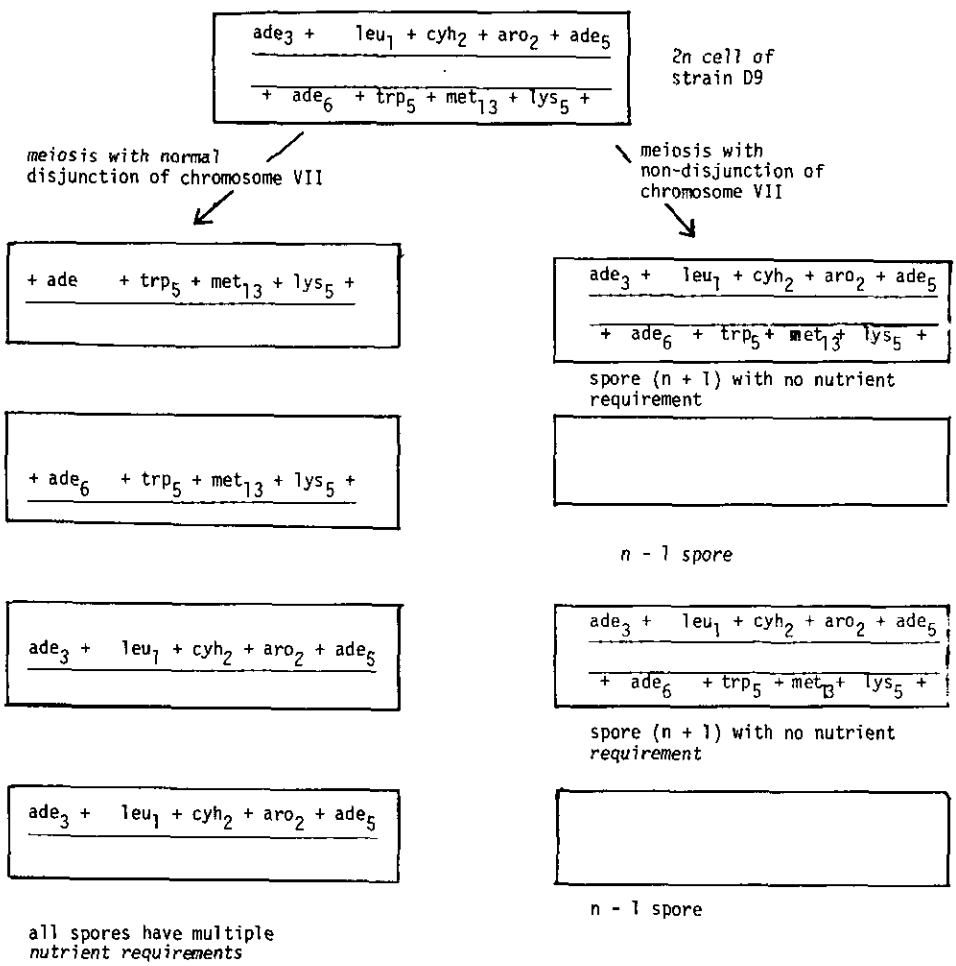

FIGURE 3. Detection of spores disomic for chromosome VII produced by nondisjunction during meiotic cell division in strain *D9*.

washed before the induction of meiosis in fresh medium, or in some cases the chemical was left for the duration of the period of meiosis and spore formation.

Treated cells of D_9J_2 were incubated on an orbital shaker at 28°C for up to 8 days to allow the cells to undergo meiosis and to produce spore tetrads. A number of chemical treatments result in a delay in spore formation and therefore the period of incubation was by necessity variable and dependant upon the observation of a yield of spore tetrads comparable to that of control cultures.

After sporulation, the cultures were washed three times in saline and incubated in 2% mushroom extract at 28°C overnight to remove the asci walls. Residual diploid cells, that had failed to undergo sporulation were killed by incubation of the cell suspension in an equal volume of diethyl ether for 10 min. The ether fraction was removed by decanting and the residual ether removed by aeration and the cells washed three times in saline. Spore suspensions were sonicated for 15 sec to assure spore separation. The remaining cell suspension was diluted and plated upon solid yeast complete medium to score viability and upon solid minimal medium plus canavanine and uracil to detect disomic spores in the cultures.

Results

The effects of treatment of yeast cells of strain *D6* with the alkylating agent ethyl methanesulfonate (EMS) followed by growth in nonselective nutrient media are shown in Figure 4. Treated stationary phase cells of *D6* were exposed to 2% EMS for periods of up to 30 min before neutralizing in 10% sodium thiosulfate before the growth period. The results in Figure 4 demonstrate that exposure to this chemical mutagen results in significant increases in the production of monosomic (white, cycloheximide resistant) colonies of at least 10 × the spontaneous frequency at exposure times greater than 20 min using procedure 1.

Figure 5 demonstrates the induction of prototrophic colonies produced by gene conversion in yeast strain *JD1* after treatment with 2% EMS in an identical method, i.e., mutagen exposure followed by 3 days growth in nonselective medium. The results obtained demonstrate that for EMS treatment, prototrophic colonies are induced at exposure times greater than 2.5 min. reaching a plateau at exposure times longer than 10 min. In contrast, monosomic colonies were induced only after considerably longer exposure times (i.e., greater than 20 min.).

Figure 6 demonstrates the effects of growth of strain *D6* in the presence of up to 40 mg/l. of mitomycin C. The results presented here, show that this crosslinking agent was effective at inducing monosomic (white, cycloheximide-resistant) colonies at concentrations as low as 4 mg/l. with maximum induction at concentrations of approximately 10 mg/l. followed by a decline at higher concentrations. Figure 7 demonstrates the effects of mitomycin C upon the induction of mitotic gene conversion in strain *JD1* under identical treatment conditions. The results obtained indicate that prototrophs produced by gene conversion were induced under similar dose response conditions to that of monosomic colonies. However, the maximum induction of prototrophs was produced at higher concentrations of mitomycin C (i.e., 20 mg/l.) than that of monosomic colonies and within the tested dose range there was no evidence of a decline in the induction of prototrophs at the higher doses.

The synthetic estrogen, stilbestrol dipropionate, was effective at inducing monosomic colonies of strain *D6* after growth in the presence of concentrations above 50 mg/l. as is shown in Figure 8. At concentrations of stilbestrol above 350 mg/l., a plateau in the induction response was observable. Similar experiments with stilbestrol dipropionate with strain *JD1* failed to produce any significant increase in the frequency of prototrophs produced by mitotic gene conversion. Under similar treatment conditions oral contraceptives containing estrogen and progesteronelike sex hormones failed to produce any increase in the frequency of monosomic colonies. Experiments performed with diethyl stilbestrol gave positive increases in the frequency of monosomic colonies in some experiments but no

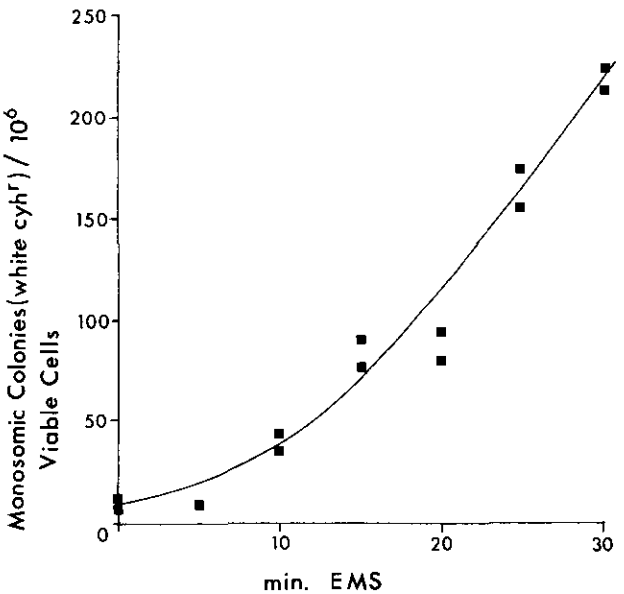

FIGURE 4. Frequency of monosomic colonies (white cyh^r) of strain *D6* produced after treatment with 2% EMS for periods of up to 30 min followed by growth in YC liquid (procedure 2).

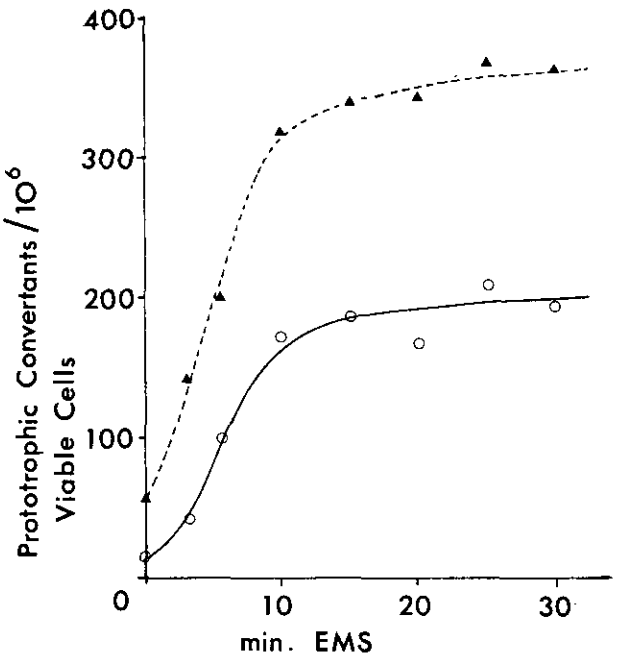

FIGURE 5. Induction of prototrophic colonies produced in strain *JD1* after treatment with 2% EMS followed by growth in YC liquid (procedure 1): (▲) trp^+ (○) his^+.

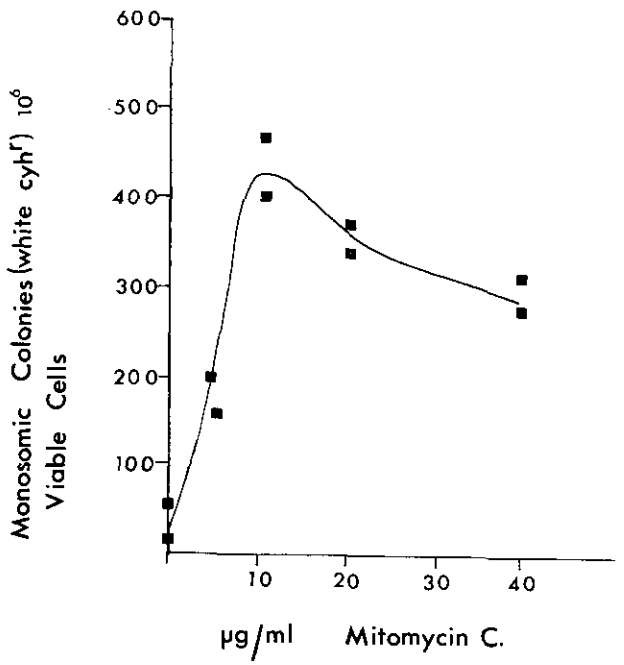

FIGURE 6. Frequency of monosomic colonies (white cyh^r) of strain *D6* produced after treatment with 0-40 μg/ml. Mitomycin C followed by growth in YC liquid (procedure 1).

response in others. At the present time we are investigating further, the reasons for the variable response obtained with this hormone.

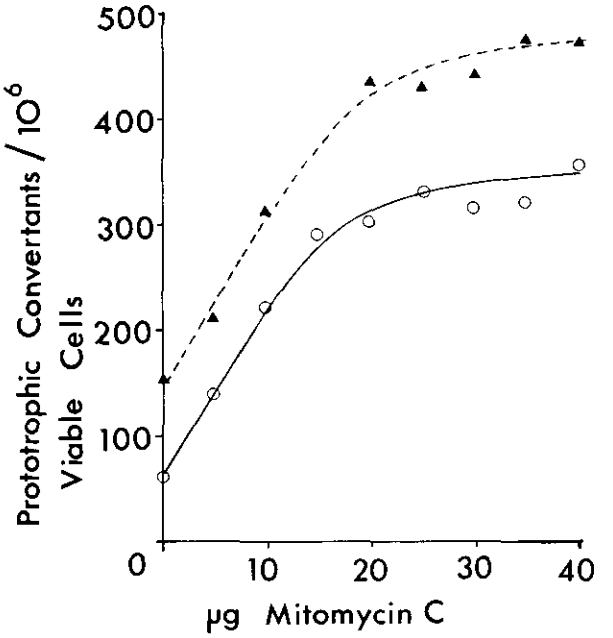

FIGURE 7. Frequency of prototrophic colonies in strain *JD1* produced by mitotic gene conversion after treatment with 0 to 40 μg/l. of mitomycin C followed by growth in YC liquid (procedure 1): (▲) trp^+; (○) his^+.

The folic acid antagonist sulfacetamide produced significant increases in monosomic colonies when cells of *D6* were grown in the presence of the drug at concentrations greater than 1000 mg/l. when we used treatment procedure 1. The results obtained, shown in Figure 9, demonstrate the production of a linear dose response curve for the induction of monosomic colonies up to concentrations of 5000 mg/l. Sulfacetamide has also been tested for its action upon *D6* by using treatment procedure 2. In this procedure (Fig. 10), the quantitative increase in the number of monosomic colonies shown in Figure 9 is reduced, but the concentration of sulfacetamide at which monosomic colonies are observed was identical (i.e., 1000 mg/l.).

A number of organic mercury compounds used as disinfectants and pharmaceutical preservatives have been tested for their effects upon induced monosomy in strain *D6*. Two of the compounds we have tested are Mercurochrome (disodium 2, 7-dibromo-4-hydroxymercurifluorescein) and Thiocid [sodium *p*-(ethyl mercurimercapto)benzene sulfonate], and the results of the tests upon these compounds are reported here.

Figure 11 shows the response of strain *D6* to growth in the presence of Mercurochrome at concentrations of 0 to 350 mg/l. by use of test procedure 1. The results obtained demonstrate the effectiveness of Mercurochrome in inducing monosomic

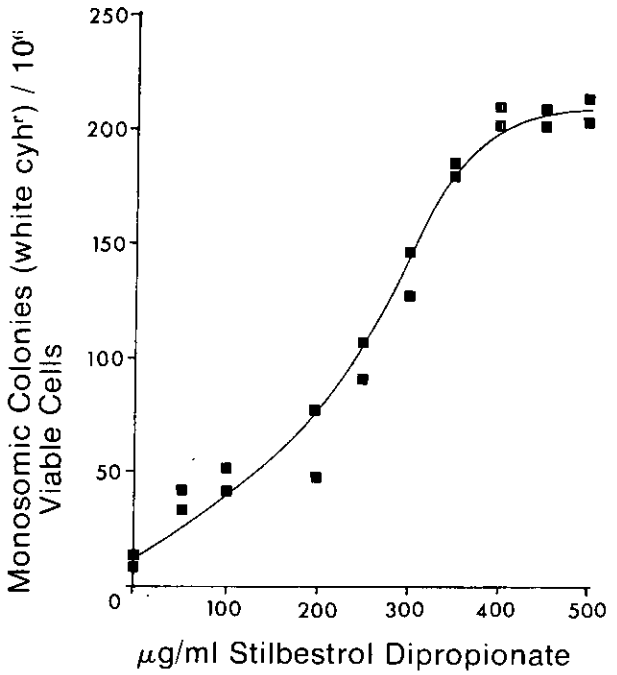

FIGURE 8. Frequency of monosomic colonies (white cyh^r) of strain *D6* produced after growth in the presence of 0 to 500 mg/l. of stilbestrol dipropionate (procedure 1).

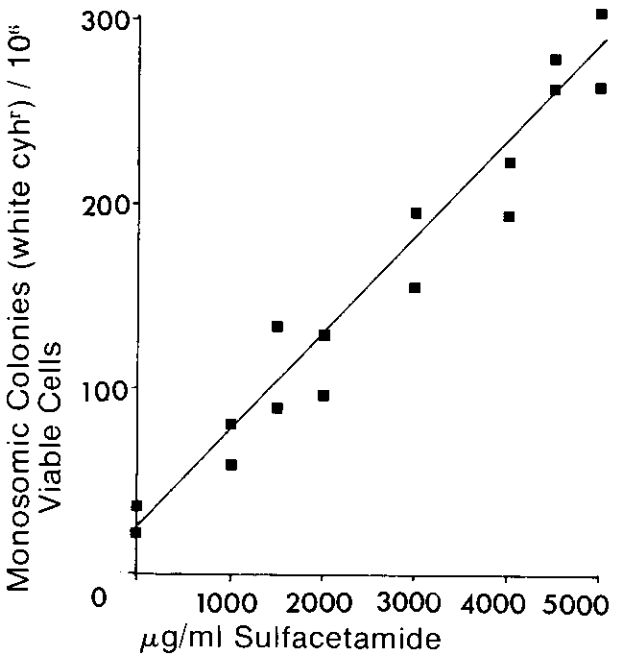

FIGURE 9. Frequency of monosomic colonies (white cyh^r) of strain *D6* produced after growth in the presence of 0 to 5000 mg/l. of sulfacetamide (procedure 1).

(white, cycloheximide-resistant) colonies at concentrations greater than 10 mg/l.; at concentrations above 100 mg/l. the yields of monosomic colonies were reduced. Experiments performed with strain *JD1* with Mercurochrome, (Fig. 12) demonstrate that the compound was also effective at inducing prototrophs produced by mitotic gene conversion.

Figure 13 demonstrates the effects of treatment of cells of yeast strain *D6* with Thiocid at concentrations of 0 to 400 mg/l. Thiocid was shown to be an effective inducer of monosomic colonies using test procedure-1 at concentrations greater than 20 mg/l. The induction curve obtained was biphasic with a change in slope at concentrations around 200 mg/l. At the present time we have no data available on the potential of Thiocid as an inducer of point mutation or mitotic gene conversion in yeast.

Figure 14 demonstrates the effects of growth of strain *D6* in the presence of up to 1000 mg/l. of caffeine in experiments by use of test procedure 2. This well known inhibitor of DNA repair activity in a variety of organisms was shown to be an effective inducer of monosomic colonies in strain *D6* at concentrations greater than 100 mg/l. Figure 14 also shows that there was no decrease in the induction of monosomic colonies over the dose range of caffeine used in these experiments.

Figure 15 demonstrates the effects of growth of strain *D6* in the presence of the synthetic sweetening agent saccharin at concentrations of 0 to 1600 mg/l. by test procedure 2. The results demonstrate that saccharin was effective at concentrations above 200 mg/l. at increasing the frequency of monosomic colonies. The results obtained suggest that the induction of monosomy would be detectable at concentrations below 200 mg/l., although at the present time no data are available on these concentrations. At concentrations of saccharin above 400 mg/l. the frequency of monosomic colonies is reduced, until at 1600 mg/l. no increase in monosomic colonies above the spontaneous value could be detected. Experiments have been performed with saccharin under identical treatment conditions with the yeast strain *D7*, which is capable of detecting mitotic crossingover and a variety of other genetic events leading to the production of aberrant colonies. No evidence of the induction of mitotic crossingover in the presence of saccharin was detectable in the experiments involving the use of *D7*.

As shown in Figure 1, the selective procedure used to detect the induction of monosomic colonies in strain *D6* involve the observation of cells white in color and resistant to cycloheximide. The presence of the other recessive alleles carried by chromosome VII requires the individual testing of these colonies on the appropriate omission media. Examples of the

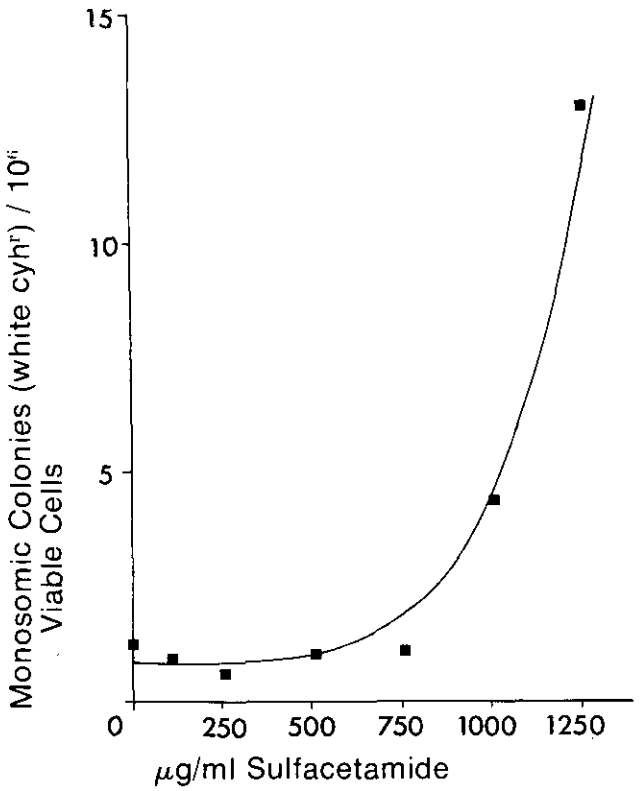

FIGURE 10. Frequency of monosomic colonies (white, cyh^r) of strain *D6* produced after growth in the presence of 0 to 1250 mg/l. of sulfacetamide (procedure 2).

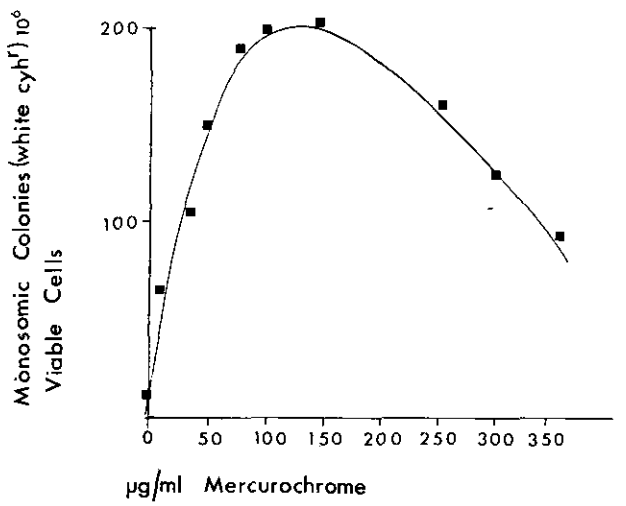

FIGURE 11. Frequency of monosomic colonies (white, cyh^r) of strain *D6* produced after growth in the presence of 0 to 350 μg/ml Mercurochrome (disodium 2,7-dibromo-4-hydroxymercurifluorescein) (procedure 1).

test procedure are shown in Table 1. The results presented are those obtained for control cultures and for cells treated with EMS and sulfacetamide, both of which were shown to be effective inducers of white, cycloheximide-resistant colonies.

Samples were taken of both white and red cycloheximide-resistant colonies obtained from selective plates of treated cells. These colonies were replica plated onto omission media lacking either leucine or tryptophan. The results shown in Table 1 clearly demonstrate that the majority of white, cycloheximide-resistant cells of *D6* were also defective in synthesis of leucine and tryptophan (71% of EMS-treated cells and 86.3% of sulfacetamide-treated cells), indicating that these cells had lost the wild type copies of these genes. In contrast, only a very small percentage of the red, cycloheximide-resistant cells (6.6% of EMS-treated cells and 6.5% of sulfacetamide-treated cells) showed similar requirements for leucine and tryptophan.

The techniques described which involve the use of selective agar containing cycloheximide were only able to detect the presence of monosomic ($2n - 1$) colonies. In contrast, trisomic ($2n + 1$) cells produced as a result of nondisjunction would not be viable on such selective medium. However, if nondisjunction occurs during the mitotic cell division of a diploid cell to produce both monosomic and

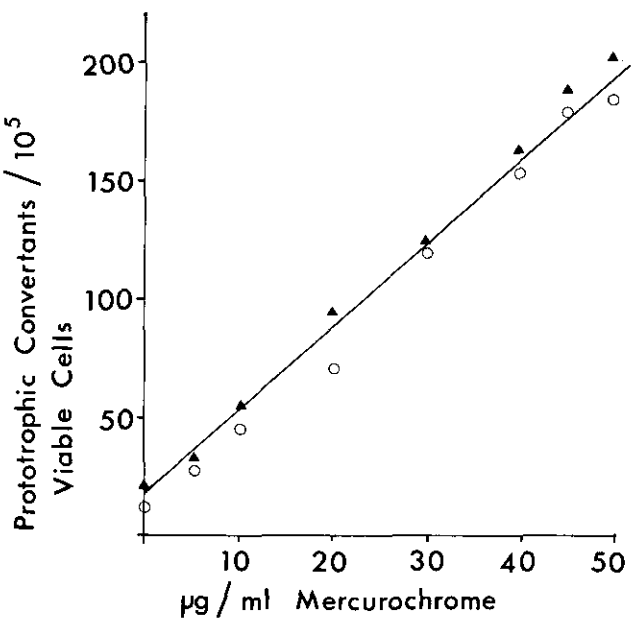

FIGURE 12. Frequency of prototrophic colonies in strain *JD1* produced by mitotic gene conversion after growth in the presence of 0 - 50 μg/ml. Mercurochrome (disodium 2,7,dibromo-4-hydroxymercurifluorescein) (procedure 2): (▲) trp$^+$; (○) his$^+$.

trisomic cells the resultant colonies should be detectable on nonselective medium by the production of half sectored red/white colonies. In such a case we would expect the red sector to be trisomic ($2n + 1$) and sensitive to cycloheximide and the white sector to be monosomic ($2n - 1$) and resistant to cycloheximide.

In our experiments we observed considerable numbers of half-sectored red/white colonies (at a frequency of approximately 0.1%). Samples of such colonies were taken and tested for the presence of trisomic red sectors by the procedure shown in Figure 16. In this procedure the white sectors of each colony were tested for cycloheximide resistance, leucine, tryptophan, methionine, adenine, and histidine requirement. In those colonies where the white sector was of the genotype $cyh^{-}leu^{-}trp^{-}met^{-}ade^{-}$ and his^{-}, the red sector was tested further. Individual red sectors were streaked onto sporulation medium, and those cultures which produced four spored asci (less than 5%) were dissected. Those asci which yielded complete tetrads (less than 10%) were replica plated and the segregation pattern of each

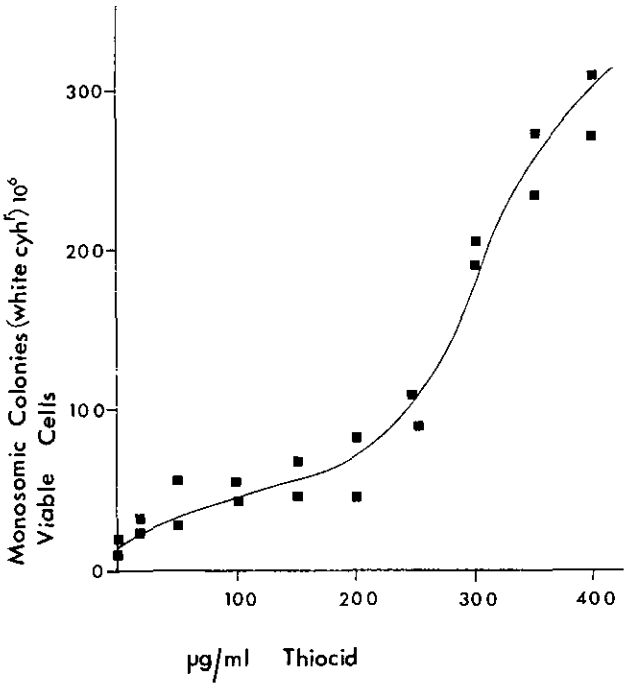

FIGURE 13. Frequency of monosomic colonies (white, cyh^r) of strain *D6* produced after growth in the presence of 0 to 400 µg/ml of Thiocid [sodium *p*-(ethylmercurimercapto) benzene sulfonate] (procedure 1).

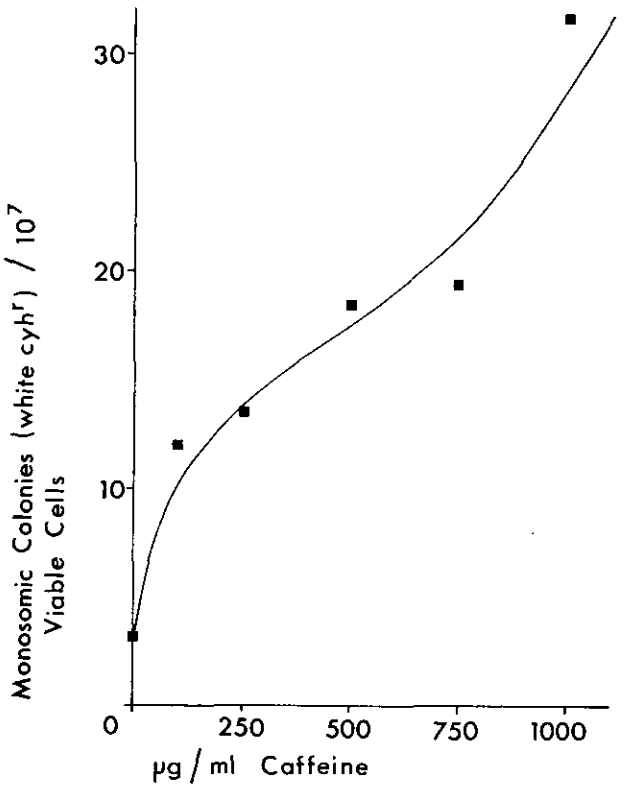

FIGURE 14. Frequency of monosomic colonies (white, cyh^r) of strain *D6* produced after growth in the presence of 0 to 1000 mg/l. caffeine (procedure 2).

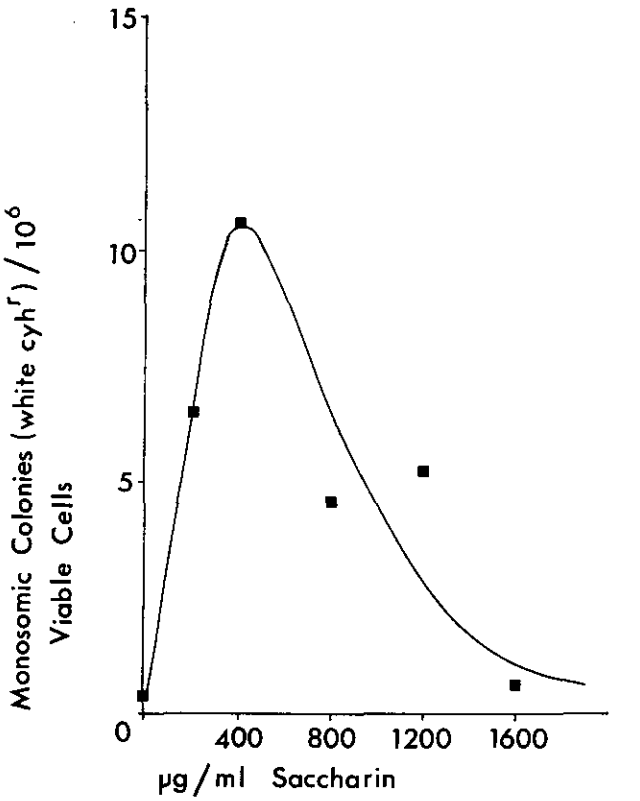

FIGURE 15. Frequency of monosomic colonies (white, cyh^r) of strain *D6* produced after growth in the presence of 0-1600 mg/l. saccharin (procedure 2).

tetrad determined. The results of these analyses are shown in Table 2 for half-sectored colonies derived from control cultures and those treated with ultraviolet light and ethyl methanesulfonate. The results obtained demonstrate that out of a total of 15 sectors studied by tetrad analysis 14 sectors showed segregation patterns indicative of a trisomic chromosome constitution. Thus at least a fraction of the half sectored colonies are made up of a monosomic sector and a trisomic sector which presumably derive from mitotic chromosome nondisjunction.

The induction of disomic spores resistant to canavanine in the strain D_9J_2 produced during meiotic cell division has so far been investigated for only one chemical agent, i.e., the amino acid analog *p*-fluorophenylalanine (PFPA). The major problem encountered with this strain has been the difficulty of distinguishing between disomic spores capable of growth on minimal medium and residual diploid cells that have not undergone meiosis and are also capable of growth on minimal medium. This difficulty is compounded by the fact that in a number of cases high concentrations of test chemicals reduce the frequency of sporulating diploid cells.

A number of techniques have been utilized in an attempt to estimate the number of disomic spores in mixed cultures of spores and diploid cells.

Selective Killing of Diploid Cells. A number of agents such as heat treatment and exposure to diethyl ether kill diploid vegetative cells at a faster rate than that of spores. Diploid cells may also be selectively killed by the induction of growth in minimal medium and the exposure of growing cells to the fungicide nystatin. Under such treatment conditions disomic spores which carry a defective allele of the *ura*$_1$ gene on chromosome XI do not grow and are not killed by nystatin exposure.

Selective Plating to Distinguish Diploid Cells and Disomic Spores. On minimal medium containing uracil growth occurs of diploid cells and spores disomic for chromosome VII. After a period of incubation at 28°C the plates can be replica plated onto minimal medium to distinguish those cells which have no requirement for the markers on chromosome VII but carry a defective allele of the *ura*$_1$ gene

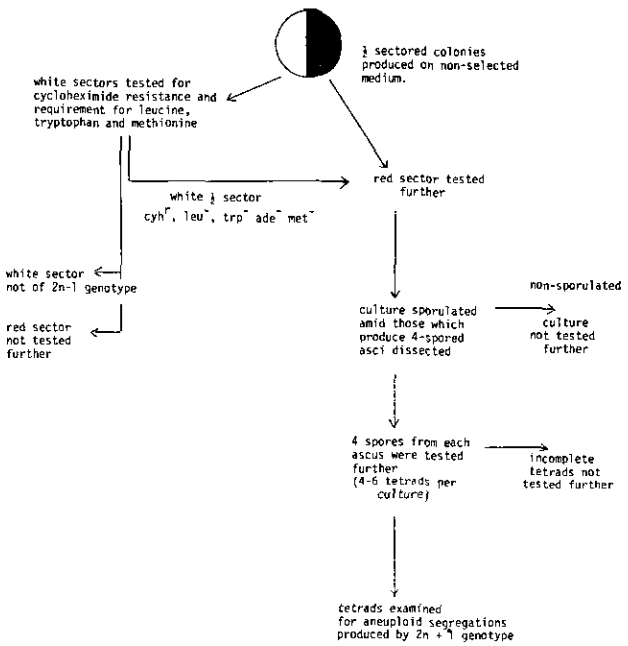

FIGURE 16. Technique used to detect trisomic ($2n + 1$) cells produced during mitotic cell division.

Table 1. Test of the phenotypes of the cycloheximide-resistant colonies of strain *D6* produced after treatment with ethyl methanesulfonate and sulfacetamide.

Treatment	Number of white cycloheximide colonies	Number of leu$^-$trp$^-$ colonies	Number of red cycloheximide-resistant cells tested	Number of leu$^-$trp$^-$ colonies
0 (Pooled data from 8 experiments)	2157	1539	3168	39
EMS (2%)				
15 min	84	59	103	7
20 min	49	32	182	12
30 min	67	51	93	6
Sulfacetamide				
150 μg/ml	104	91	114	3
3000 μg/ml	153	138	86	7
5000 μg/ml	86	67	172	14

on chromosome XI (50% of the spores disomic for chromosome VII. The heterozygous condition of the *can-1* gene carried on chromosome V results in the sensitivity of diploid cells of strain D_9J_2 to the presence of the drug caravanine in the growth medium (50% of the disomic spores produced are also sensitive to caravanine). Plating of mixed culture of diploid cells and spores of D_9J_2 upon minimal medium containing uracil and caravanine results in the growth on this medium of disomic spores carrying the recessive allele *can-1* conferring resistance to caravanine.

Gradient Separation of Diploid Cells and Spores. Centrifugation of mixed cell suspensions in sucrose gradients results in a separation of cells based upon cell volume. In view of the large size differences between diploid cells and spores they may be readily separated from each other by centrifugation techniques. The large-scale separation of spores and diploid cells has been undertaken in our laboratory by using a zonal rotor (R. S. Tippins, personal communication). A major advantage of this technique is that even in those cultures which produce only a low percentage of spores repeated gradient separation on a zonal rotor can result in the production of large numbers of spores with little or no contamination from residual diploid cells.

The increases of disomic spores resistant to canavanine observable after the meiotic cell division of cells of D_9J_2 treated with PFPA are shown in Figure 17 and for comparison after treatment with ultraviolet light in Figure 18. In experiments using both treatments the cells were exposed to diethyl ether after sporulation as described in the Materials and Methods.

Ultraviolet light exposures greater than 10 J/m^2 produce increases in the frequency of disomic spores, at doses greater than 500 J/m^2 the induction curve reaches a plateau, although at these doses the frequency of diploid cells undergoing meiosis was significantly reduced which prevents the accurage assay of disomic spores at these high doses. PFPA treatment which involved the drug being present during the period of spore formation was effective in inducing disomic spores at concentrations above 50 mg/l. at concentrations from 300 to 550 mg/l. the drug produces an exponential increase in the frequency of disomic spores although at the higher doses the frequency of sporulating cells was reduced. However, in the case of both ultraviolet light and PFPA treatments the frequencies of disomic spores resistant to caravanine could be assayed with precision at the lower end of the dose range where the majority of the cells undergo sporulation to produce spore tetrads.

Discussion

The results presented demonstrate that it is a practical possibility to utilize yeast cultures to study the ability of environmental chemicals to induce aneuploidy during either mitotic or meiotic cell division. The ability of yeast cells to undergo such

Table 2. Detection of trisomic ($2n + 1$) colonies produced mitotic cell division after mutagen treatment of cells of strain *D6* (half sectored red/white colonies identified on nonselective medium were tested).

Treatment	No. of half red sectors	Sectors	No. of tetrads tested	No. of tetrads showing aneuploid segregation
Control	5	1	6	5
		2	5	5
		3	6	5
		4	5	5
		5	5	5
UV light (samples taken from doses of up to 500 J/m^2)	5	1	5	5
		2	4	4
		3	5	4
		4	6	6
		5	5	5
Ethyl methanesulfonate, 2% (samples taken from treatment time of 15 to 30 min)	5	1	5	5
		2	5	5
		3	5	4
		4	5	5
		5	3	0

changes in chromosome number has been shown to take place in the presence of a number of chemicals. In view of the potential genetic consequences of such changes in chromosome number such activity may represent a previously underestimated hazard in the use of a variety of chemicals. We suggest that the ability of an agent to induce aneuploidy might well be given a name and we consider that "aneugenic" might well be an appropriate term.

Monosomic colonies are induced in yeast cultures undergoing mitotic cell division by a variety of well-known mutagens of widely different modes of action. In this paper we present evidence that the alkylating agent ethyl methanesulfonate and the crosslinking agent mitomycin C induce monosomic colonies at high frequencies. Similar results have been obtained for the related alkylating agent methyl methanesulfonate (D. Sharp, unpublished data). Both mitomycin C and EMS have also been shown to be effective inducers of mitotic gene conversion under similar experimental conditions (*29*).

Organic mercury preservatives are widely used in the prevention of bacterial contamination of pharmaceutical products. We show here that two organic mercury preservatives, Mercurochrome and Thiocid, are effective inducers of monosomic colonies in yeast cells undergoing mitotic cell division. Mercurochrome was also shown to be an inducer of mitotic gene conversion in yeast cells treated under the same conditions. A related organic mercury preservative thiomersal has been previously shown by us to be an effective inducer of mitotic gene conversion in yeast at concentrations as low as 0.001 mg/l. (*30*).

The other chemicals shown in the results presented here to be inducers of monosomic cells in yeast do not induce mitotic gene conversion or mutation in yeast. These compounds, i.e., stilbestrol dipropionate, sulfacetamide, caffeine, and saccharin could appropriately be described as aneugenic in yeast and would not be detectable as genetically active in the more generally used microbial assay systems.

The synthetic estrogen, diethylstilbestrol, has been linked with the induction of cancer of the vagina in the adolescent female offspring of treated mothers (*31*) and has been shown to produce aneuploidy in the bone marrow cells of the mouse (*32*). Recent works (*33*) with mammalian cells in culture suggests that diethylstilbestrol produces polyploidy and is similar in action to colchicine in treated cells. Diethylstilbestrol and the related compound stilbestrol

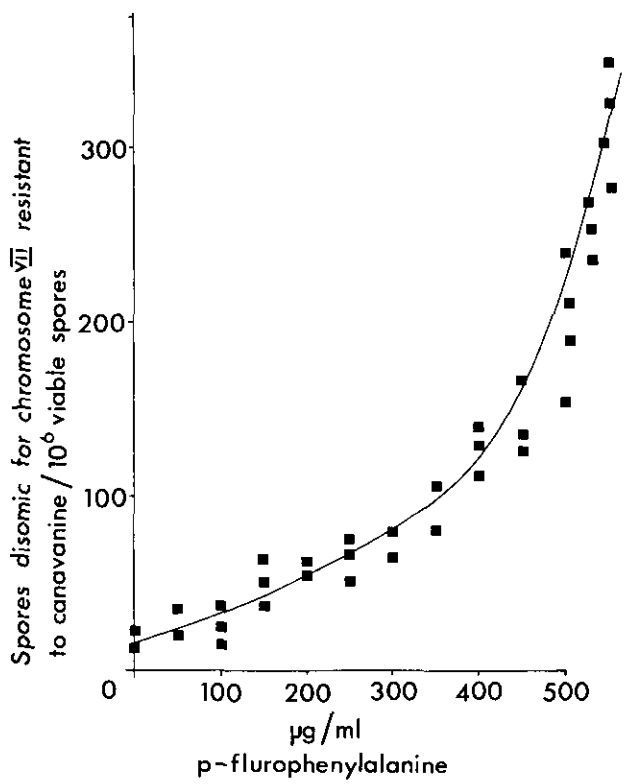

FIGURE 17. Frequency of spores disomic for chromosome VII in strain D_8J_2 after treatment with 0 to 550 mg/l. *p*-fluorophenylalanine.

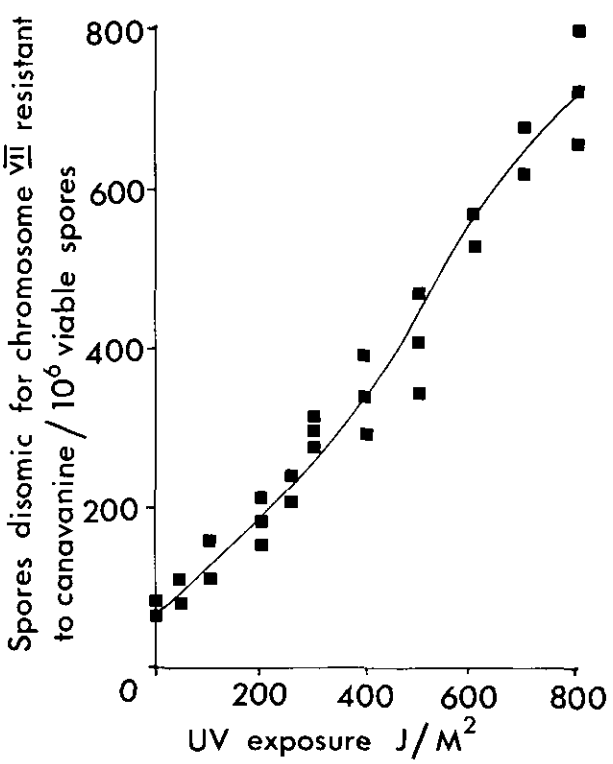

FIGURE 18. Frequency of spores disomic for chromosome VII in strain D_8J_2 after exposure to ultraviolet light 0 to 800 J/m².

dipropionate have been used for the treatment of menopausal symptoms, suppression of lactation, as an abortifacient and in the treatment of prostrate and mammary carcinomas. At the biochemical level diethylstilbestrol has been shown to bind with DNA *in vitro* as a result of the action of ultraviolet irradiation or oxidation (*34*).

The sulfa drugs, such as sulfacetamide, are antibacterial in action, probably due to their ability to act as false substrates for *p*-aminobenzoic acid during folic acid synthesis (*35*). Although largely superceded by antibiotics, sulfonamides such as sulfamethoxazole are still used in conjunction with other folic acid antagonists such as trimethoprin in the treatment of urinary tract and respiratory infections.

Saccharin at high dose levels has been demonstrated to produce tumors in experimental animals (*36*). However, there is no convincing evidence that pure saccharin is capable of inducing mutation in bacterial cultures, and it has recently been suggested that saccharin acts as a carcinogen by some epigenetic mechanism (*37*). It is of considerable interest that saccharin treatment results in the induction of monosomic colonies in yeast. However, at this stage it would be premature to speculate on the possible role of induced aneuploidy (aneugenic mechanism) on the production of tumors after saccharin treatment.

The screening procedure for induced monosomy using yeast cultures such as *D6* and its derivatives can be readily incorporated into a battery of microbial test systems used in the detection of mutagenic and carcinogen chemicals. The testing of a chemical for such activity can be performed over a period of approximately 4 weeks at a cost of $1500. The demonstration that at least a percentage of the monosomic colonies induced in *D6* are produced by mitotic chromosome nondisjunction requires a high level of skill and is probably inappropriate for routine testing.

The demonstration of the production of spores disomic for chromosome VII in the yeast strain D_9J_2 after treatment with *p*-fluorophenylalanine and ultraviolet light show the practicality of such strains of yeast for the study of environmentally induced chromosome nondisjunction during meiosis. However, at the present time there are still a number of technical difficulties to overcome before the system can be used in a routine manner for the detection of meiotic nondisjunction.

The induction of aneuploidy during meiosis in the prensence of *p*-fluorophenylalanine was not unexpected as there is a long history of the use of *p*-fluorophenylalanine to induce aneuploidy in a variety of fungal cultures (*18, 38, 39*) and we have recently demonstrated that growth in the presence of *p*-fluorophenylalanine also results in the induction of mitotic gene conversion in yeast (*40*). However, the chemical has proved to be useful in our work as a standard for the evaluation of suitable yeast strains and technical modifications necessary for the detection of induced meiotic aneuploidy in yeast.

In view of the mechanistic differences that may exist in the induction of aneuploidy during mitosis and meiosis it is now appropriate that those chemicals which are positive aneugens in mitotically dividing cells be tested in the meiotic yeast system described here. Clearly the response of both mitotic and meiotic yeast cells to *p*-fluorophenylalanine suggest that the simple mitotic system may detect at least a proportion of the chemicals active at meiosis.

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